REMARKS / ARGUMENTS

Claims 1-43 are pending in the instant application. In a Response to Restriction Requirement earlier filed on November 22, 2005, Applicants elected without traverse to pursue prosecution of Group I, claims 1-26. Applicants hereby affirm the election with traverse of Group I, claims 1-26. Claims 1-26 are amended by the present Amendment. Upon entry of the present Amendment, claims 1-43 are pending and presented for reconsideration. Applicants respectfully submit that no new matter is introduced by the present Amendment.

Amendment and/or cancellation of the claims is not to be construed as acquiescence to any of the objections/rejections set forth in the instant Office Action or any previous Office Action of the parent application, and was done solely to expedite prosecution of the application. Applicants submit that claims were not added or amended during the prosecution of the instant application for reasons related to patentability. Applicants reserve the right to pursue the claims, as originally filed, or similar claims in this or one or more subsequent patent applications.

Claim Rejections - 35 U.S.C. §112

Rejection of Claims 1-26 under 35 U.S.C. §112, Second Paragraph

Claims 1-26 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In particular, the Office Action, on page 3, states that the term "function" is indefinite because "it is not clear what is meant by a recombinase 'function.'"

Without acquiescing to this rejection and solely in an effort to further prosecution, Applicants have amended claims 1-2, 4-5, and 7-14 to remove the word "function." Applicants, therefore, respectfully request withdrawal of the rejection of claims 1-2, 4-5, and 7-14, and therefore dependent claims 3, 6, and 15-26, under 35 U.S.C. §112, second paragraph and favorable reconsideration.

Rejection of Claims 1-2, 4-5, and 7-14 under 35 U.S.C. §112, Second Paragraph

Claims 1-2, 4-5, and 7-14 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In particular, the Office Action, on page 3, states that it is "unclear how many sequences are encompassed."

Without acquiescing to this rejection and solely in an effort to further prosecution, Applicants have amended claims 1-2, 4-5, and 7-14 to specifically refer to "*two* nucleotide sequences" or "*a* nucleotide sequence," where applicable. Applicants, therefore, respectfully request withdrawal of the rejection of claims 1-2, 4-5, and 7-14, under 35 U.S.C. §112, second paragraph and favorable reconsideration.

Claim Rejections - 35 U.S.C. §103

Rejection of Claims 1, 4, and 7-8 under 35 U.S.C. §103(a)

Claims 1, 4, and 7-8 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Stewart *et al.* in view of Poteete *et al.* In particular, the Office Action states that, "there would have been a reasonable expectation of success to result when combining the isolated nucleic acid comprising phage λ *bet*, *exo*, and *gam* genes under the control of the P*tac* promoter as taught by Poteete *et al.* in the nucleic acid of Stewart *et al.* comprising λ *bet* and *exo* sequences as well as the *lacI* gene under the control of its native promoter."

Applicants respectfully traverse the Examiner's assertion that the claimed invention would have been obvious to the skilled artisan at the time it was made. Reconsideration and withdrawal of the rejection in light of the following discussion is respectfully requested.

To establish a *prima facie* case of obviousness, it is necessary for the Examiner to present evidence, preferably in the form of some teaching, suggestion, incentive or inference in the applied references, or in the form of generally available knowledge, that one having ordinary skill in the art would have been motivated to make the claimed invention and would have had a reasonable expectation of success in making the claimed invention. Under section 103, "[b]oth the suggestion and the expectation of success must be founded in the prior art, not in applicant's disclosure" (*Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.* 927 F.2d 1200, 1207, 18 USPQ2d

1016 (Fed. Cir. 1991), quoting *In re Dow Chemical Co.,* 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed Cir. 1988)). Moreover, when a combination of references are used to establish a *prima facie* case of obviousness, the Examiner must present evidence that one having ordinary skill in the art would have been motivated to combine the teachings in the applied references in the proposed manner to arrive at the claimed invention. See, e.g., *Carella v. Starlight Archery*, 804 F.2d 135, 231 USPQ 644 (Fed. Cir. 1986); and *Ashland Oil, Inc. v. Delta Resins and Refractories, Inc.*, 776 F.2d 281, 227 USPQ 657 (Fed. Cir. 1985).

Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness since the cited references would not have motivated one of ordinary skill in the art to arrive at the claimed invention.

The present invention features improved methods and systems for promoting recombination in bacteria, specifically in pathogenic strains of bacteria. The Office Action states that "it would have been obvious for one of ordinary skill in the art to extend the use of such nucleic acids to hosts such as (entero)pathogenic *E. coli, Mycobacterium tuberculosis*, and *Pseudomonas aeruginosa*..." Contrary to this statement, there was no reasonable expectation of success in achieving recombination in pathogenic strains at the time of the claimed invention. At the time of the instant invention, ***the known λ Red systems, i.e., the λ Red taught by Datsenko et al., Poteete et al., or Stewart et al., could not be used successfully in pathogenic bacterial strains***. For example, as described in the instant application on page 39, lines 17-20, "***initial attempts by the instant inventors to employ λ Red,"*** **(*i.e.*, the λ Red taught by Datsenko *et al.*, Poteete *et al.*, or Stewart *et al.*)**, ***"for PCR-mediated gene replacement at various loci in [the pathogens] EHEC and EPEC were met with sporadic success***, similar to the limited success seen with Red-promoted short homology recombination in *Y. pseudotuberculosis*." It was these difficulties that prompted the inventors to examine more closely the methodologies of λ Red promoted PCR-mediated gene replacement, especially in regard to optimizing its use in the pathogenic organisms EHEC and EPEC. The specification further states on page 53, lines 26-28, that, in contrast to known λ Red systems, "[t]he above Examples demonstrated that λ Red can be utilized for the manipulation of the chromosomes of [the pathogens] EHEC and EPEC" and that "the ability to inactivate or replace a gene of interest in the chromosomes of bacterial pathogens is a critical step in the identification of virulence factors." To summarize, at the time of the instant

invention, the use of known λ Red systems, including the λ Red taught by Datsenko *et al.*, Poteete *et al.*, or Stewart *et al.*, for PCR-mediated gene replacement in pathogenic species led only to inefficient and sporadic results. ***The limited success of the previous λ Red systems (i.e., the λ Red taught by Datsenko et al., Poteete et al., or Stewart et al.), led the inventors to select the specific combination of recombinase, anti-recombinase, Ptac promoter, and lacI presently claimed to improve efficiency of Red recombineering in pathogenic strains.***

Stewart *et al.* teach an isolated nucleic acid comprising the recombinase λ Red α (*exo*) and Red β (*bet*) genes. Stewart *et al.* teach that levels of expression can also be varied by using promoters of different strengths, including but not limited to, the araC promoter, the p_L promoter, the trp promoter, the T7 gene-10 promoter, phoA, recA, and the tac promoter. Stewart *et al.* specifically teach that "the tac promoter... will result in high baseline levels of expression, and should be used only when overexpression is required." Independent of the promoter selection, Stewart *et al.* teach inducible expression of the recombinase by utilizing a variety of inducible regulatory sequences. Specifically, Stewart *et al.* teach that, "in one embodiment, for example, the lacI gene and its gratuitous inducer IPTG can be utilized to yield inducible, high levels of expression." According to the reference, these recombinase sequences may be encoded in a multi-copy plasmid or in single-copy in the chromosome. As acknowledged by the Examiner on page 6 of the Office Action, this reference is devoid of any teaching regarding "an isolated nucleic acid comprising sequences encoding an anti-recombinase function such as λ Red γ (*gam*) under the control of a P*tac* promoter."

The Poteete reference discloses a nucleic acid comprising the Red genes (*gam, bet,* and *exo*) of phage λ under the control of a P*tac* promoter, ***inserted into the chromosome in place of the recBCD gene cluster*** (see Abstract), for use in a method of homologous recombination within *Escherichia coli*. However, the Poteete reference fails to teach or suggest an ***isolated*** nucleic acid, *e.g.*, a nucleic acid encoded in a plasmid or vector as presently claimed. The instant invention teaches in the specification, on page 7, lines 16-17, that "isolated" nucleic acid molecules, by definition, are nucleic acid molecules which are separated from the chromosome in which the genomic DNA is naturally associated. Furthermore, the instant invention on page 8, lines 30-32, teaches that an inducible promoter, "directs expression of a gene where the level of expression is alterable by environmental or developmental

factors such as, for example, temperature, pH, transcription factors, activators, repressors, and chemicals." The expression of nucleic acid sequences (*e.g.*, recombinase, anti-recombinase, or *lacI*) in bacteria (*e.g.*, EHEC or other pathogenic bacteria) is sensitive to several additional factors, including the number of copies of the sequence in the cell and the coiling of the DNA. Differences in copy number and coiling can often lead to differential expression and/or regulation of the same nucleic acid sequence. For example, a nucleic acid sequence present as an isolated nucleic acid molecule (*e.g.*, a plasmid) or the same nucleic acid sequence present in a chromosome may be expressed differently. The specific constructs presently claimed allow for the intricate regulation and expression of the recombinase nucleic acid sequences and result in levels of expression that promote efficient recombination in pathogenic bacteria. The construct disclosed in Poteete *et al.* is inserted into the chromosome, and therefore one of ordinary skill in the art would not have been motivated to combine certain elements of such a chromosomal construct with that taught by Stewart *et al.* for use as an isolated molecule. Moreover, in light of the differential expression and/or regulation of genes between an isolated nucleic acid sequence (*e.g.*, a plasmid) and nucleic acid sequences encoded in the chromosome, there was no reasonable expectation of success at the time of the claimed invention in combining certain elements of the isolated nucleic acid molecule of Stewart *et al.* with elements of the chromosomal nucleic acid molecule of Poteete *et al.*

In summary, Applicants respectfully submit that, contrary to the Examiner's assertions, the ordinarily skilled artisan at the time of Applicants' invention would not have been motivated nor have reasonably expected to succeed in arriving at Applicants' invention based on the teachings of Poteete *et al.* and/or Stewart *et al.* The claimed invention pertains to isolated nucleic acid molecules comprising the recombination genes of phage λ *exo*, *bet* and *gam* operably linked to a P*tac* promoter sequence, and the use of a *lacI* gene in order to induce expression of the recombinase. These molecules are not taught or suggested in the cited art. Therefore, Applicants respectfully request withdrawal of the rejection of claims 1, 4, and 7-8 under 35 U.S.C. §103(a) and favorable reconsideration.

Rejection of Claims 1-26 under 35 U.S.C. §103(a)

Claims 1-26 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Stewart *et al.* in view of Poteete *et al.* and further in view of Datsenko *et al.* In particular, the Office Action states that, "[i]t would have been obvious for one of ordinary skill in the art to use the temperature sensitive origin of replication of Datsenko et al with the nucleic acid of Stewart et al and Poteete et al because all three references teach efficient recombination in E. coli with the use of nucleic acids comprising λ Red sequences...", and because they "teach that such an addition [of a temperature sensitive origin of replication] allows for easy elimination of the vector." Additionally, the Office Action states that "it would have been obvious for one of ordinary skill in the art to extend the use of such nucleic acids to hosts such as (entero)pathogenic *E. coli, Mycobacterium tuberculosis*, and *Pseudomonas aeruginosa*."

Applicants respectfully traverse the Examiner's assertion that the claimed invention would have been obvious to the skilled artisan at the time it was made. Reconsideration and withdrawal of the rejection in light of the following discussion is respectfully requested.

The legal requirements to establish a *prima facie* case of obviousness are set forth above. Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness since at the time the invention was made there was no motivation to combine the references in the manner suggested by the Examiner, nor was there a reasonable expectation of success in making the claimed invention. The teachings of Stewart *et al.* and Poteete *et al.* are set forth above. As discussed previously, the Examiner has not provided the requisite motivation to combine the Stewart and Poteete references. In addition, based on the teachings of the references, there was no reasonable expectation of success in making the claimed invention based on the teachings of these references. Datsenko *et al.* teach the use of an isolated nucleic acid comprising λ *bet, exo* and *gam* sequences under the control of an arabinose-inducible promoter (P_{bad}) in a low copy number vector (*i.e.,* isolated nucleic acid molecule), further comprising a temperature sensitive origin of replication, allowing for easy elimination of the vector at 37°C, with an optimized ribosome-binding site for efficient translation of *gam*. This reference is devoid of any teaching, regarding an isolated nucleic acid comprising sequences encoding λ Red Recombinase sequences under the control of a P*tac* promoter or a *lacI* gene operably linked to its native

promoter. Similarly, this reference fails to provide the motivation required to modify the teachings of the cited art to arrive at the claimed invention. Absent the teachings of the instant application there was no motivation to make the claimed specific constructs which consistantly allow for efficient recombination in pathogenic organisms. More specifically, there was no motivation to combine a P_{tac} promoter, recombinase sequences, an anti-recombinase sequence, and an origin of replication which confers low copy number and temperature sensitivity in an isolated nucleic acid molecule.

In summary, Applicants respectfully submit that, contrary to the Examiner's assertions, the ordinarily skilled artisan at the time of Applicants' invention would not have been motivated nor have reasonably expected to succeed in arriving at Applicants' invention based on the teachings of Datsenko *et al.*, Poteete *et al.*, and/or Stewart *et al.* Therefore, the claimed invention is not obvious in view of the cited art. Applicants respectfully request withdrawal of the rejection of claims 1-26 under 35 U.S.C. §103(a) and favorable reconsideration.

CONCLUSION

In view of the foregoing, entry of the amendments and remarks presented, favorable reconsideration and withdrawal of the rejections, and allowance of this application with the pending claim are respectfully requested. If a telephone conversation with the Applicant's attorney would expedite prosecution of the above-identified application, the Examiner is invited to call the undersigned at (617) 227-7400.

Respectfully submitted,

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